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13. ABSTRACT (Maximum 200 Words)

Breast cancer (BC) is a heterogeneous disease with varying clinical behavior, and response to therapy that cannot be predicted based on clinical and pathologic classifications. It is the primary goal of our research to identify and characterize biological pathways and individual molecular components that play a primary role in BC development and progression. In order to identify genes, gene expression profiles and molecular pathways associated with metastatic BC we have performed genome-wide gene expression analysis of a large number of breast cancer samples. Both unsupervised and supervised analyses are being used to identify genes differentially expressed among samples. Hierarchical clustering showed that most samples grouped according to estrogen receptor status. In addition, matched primary carcinomas and lymph node metastases tended to pair demonstrating marked conservation of molecular phenotype within patients. Formal statistical testing is being used to identify genes with marked changes in expression during progression. Lymph node metastases in particular showed significant decreases in the expression of many genes corresponding to extracellular matrix proteins and proteases when compared to matched primaries. Further expression changes in a variety of genes were associated with distant metastases. Immunohistochemistry and in situ hybridization are being used to validate and extend findings.

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Table of Contents

INTRODUCTION

Breast cancer (BC) is a heterogeneous disease with varying clinical behavior and response to therapy that cannot be predicted based on existing clinical and pathologic classifications. This has led to an intense effort to understand the biology of BC and a search for genes and gene products that play a major role in tumor development and progression. A comprehensive analysis of gene expression can provide crucial clues concerning the intrinsic biology of a cancer and ultimately contribute to diagnostic decisions and therapies tailored to an individual patient. New, high-throughput mRNA analysis platforms, such as DNA microarrays, allow comprehensive measurement of gene expression and can produce large data sets with the potential to provide novel insights into biology at the molecular level. Our studies are designed to identify gene expression profiles that are associated with tumor progression and can be used for discrimination of clinically relevant subgroups of BC. An understanding of the mechanisms that drive progression of BC will provide biomarkers for diagnosis, risk stratification and therapeutic targets that could have an enormous impact on the care of these patients. The specific aims of our project are: 1) To identify the genes, gene expression profiles and molecular pathways associated with metastatic BC using microarray based, gene expression analysis and comparison of concurrent primary and metastatic tumors within the same patients. 2) To identify gene expression differences associated with clinical outcome by comparison of comprehensive expression profiles from stage and histology matched primary BCs in patients with long term recurrence-free survival and patients that die of metastatic disease.

BODY

Task 1 To identify the genes, gene expression profiles and molecular pathways associated with metastatic breast cancer using microarray based, gene expression analysis and comparison of concurrent primary and metastatic tumors within the same patients

a. Evaluation and selection of tumor cases to be used (months 1-6)
b. Microdissection of frozen tissue, RNA preparation and analysis. (months 3-9)
c. Microarray screen and analysis of data (months 6-12
d. Characterization of differentially expressed genes (months 9-24)
e. Design and optimization of methods for molecular testing in additional samples (quantitative RT-PCR, in situ hybridization, etc). (months 18-30)
f. Testing of additional cases and data analysis (months 24-36)

We have completed all tasks originally proposed for the first two years of funding. Specifically we have identified and processed all tissue samples planned for specific aims 1 and 2. RNA has been isolated and labeled cRNA target from these samples has been subjected to gene expression analysis using oligonucleotides microarrays with features for over 33000 genes/ESTs. Hierarchical clustering of the gene expression data showed that most samples grouped according to estrogen receptor status (ER). In addition, the matched primary carcinomas and lymph node metastases have global expression profiles more similar to each other than to other breast cancers. Formal statistical testing identified genes that had marked changes in expression during progression. In particular lymph node metastases showed significant decreases in the expression of many genes corresponding to extracellular matrix proteins and proteases when compared to matched primaries. Very few genes were overexpressed in lymph node metastases compared to the corresponding primary.

In an effort to further characterize the expression and distribution of differentially expressed extracellular matrix associated genes, we used immunohistochemistry in tissue sections of samples used for expression analysis. Immunohistochemistry for MMP2, MMP3 and MMP11 showed a similar distribution with predominant reactivity in tumor cells. Several matched pairs demonstrated a reduction of MMP in lymph node metastases consistent with the RNA levels. Decorin, an extracellular matrix constituent that interacts with epidermal growth factor and has anti oncogenic activity was expressed in the majority of tumors predominantly in tumor cells. Although levels of reactivity were in general similar between matched pairs several individual cases demonstrated a decreased level of decorin in the lymph node metastases. Immunoreactivity for FBLN1 was present in almost all breast cancer samples primarily in cancer cells and variably present in adjacent extracellular matrix.

Task 2 To identify gene expression differences associated with clinical outcome by comparison of comprehensive expression profiles from stage and histology matched primary breast cancers in patients with long term recurrence-free survival and patients that die of metastatic disease.

a. Evaluation and selection of tumor cases to be used (months 3-9)
b. Microdissection of frozen tissue, RNA preparation and analysis. (months 6-12)
c. Microarray screen and analysis of data (months 9-15
d. Characterization of differentially expressed genes (months 12-24)
e. Design and optimization of methods for molecular testing in additional samples (quantitative RT-PCR, in situ hybridization, etc). (months 18-30)
f. Testing of additional cases and data analysis (months 24-36)

For specific aim 2, we have identified, processed and performed expression analysis for 72 early stage primary breast cancers with appropriate outcome data that are matched for stage and treatment. Forty-six have no recurrence with a median follow-up of about 7 years and 26 have had a distant recurrence. We have performed an initial analysis and identified genes with strong and consistent differential expression between outcome groups (Table 1.). It is interesting that several of these genes are believed to participate processes that may contribute to tumor biology and deserve additional study. Confirmation of these results and their use to develop predictive models is underway.

TABLE 1

Gene Title	Chromosomal Location	GO Molecular Function Description
sterol regulatory element binding transcription factor 1 /// sterol regulatory element binding transcription factor 1	17p11.2	transcription factor activity /// RNA polymerase II transcription factor activity
programmed cell death 4 (neoplastic transformation inhibitor)	10q24	---
zinc finger protein 238	1q44-qter	DNA binding /// protein binding /// zinc ion binding
immunoglobulin heavy constant mu	14q32.33	antigen binding
block of proliferation 1	8q24.3	---
programmed cell death 4 (neoplastic transformation inhibitor)	10q24	---
transport-secretion protein 2.2	11p15.5	catalytic activity /// nutrient reservoir activity

zinc finger protein 238	1q44-qter	DNA binding /// protein binding /// zinc ion binding
hypothetical protein LOC92482	10q25.3	---
phosphatidylinositol (4,5) bisphosphate 5-phosphatase, A	22q11.2-q13.2	inositol/phosphatidylinositol phosphatase activity /// inositol-polyphosphate 5-phosphatase activity /// hydrolase activity
ornithine decarboxylase antizyme 3	1q21.3	ornithine decarboxylase inhibitor activity /// ODC_AZ;enzyme inhibitor activity;9.3e-08
cytochrome c oxidase subunit Vb	2cen-q13	cytochrome-c oxidase activity /// oxidoreductase activity

KEY RESEARCH ACCOMPLISHMENTS

1) Evaluation and selection of tumor cases to be used for specific aims 1 and 2
2) Microdissection of frozen tissue, RNA preparation and analysis of all samples.
3) Microarray based gene expression analysis of all samples.
4) Analysis of data from specific aims 1 and 2 and identification of differentially expressed genes.
5) Validation of differential expression at the RNA and protein level for select genes.

REPORTABLE OUTCOMES

Comprehensive gene expression analysis of paired primary breast carcinomas and lymph node metastases. MCV. Donaton, D Giri, A Olshen, K Panageas, S Levcovici, P Lal, E Brogi, C Hudis, K VanZee, L Tan, W Gerald. Abstract presentation American Association of Cancer Research, 2003.

Gene expression differences between paired primary and metastatic breast carcinomas. D Giri, M Donaton, A Olshen, K Panageas, P Lal, S Levcovici, E Brogi, C Hudis, K VanZee, L Tan, W Gerald. Abstract presentation United States and Canadian Academy of Pathology, 2003.

CONCLUSIONS

Comprehensive gene expression analysis of archived breast cancer samples is feasible. Molecular subgroups of breast carcinoma identified by gene expression analysis are strongly influenced by the ER status of the tumor. The gene expression profiles of paired primary and metastatic breast carcinomas are remarkably similar and the differences observed appear to reflect different microenvironments and tissue specific responses to tumor growth. Taken together, these results suggest that molecular features of breast carcinomas metastatic to lymph nodes are largely present in the primary tumor and might have been acquired early in tumorigenesis.

Analysis of primary tumors from patients with differing outcomes demonstrated a relatively small number of genes associated with progression. However several have interesting functional attributes that could impact on tumor biology. Further study is in progress.

REFERENCES

None

APPENDICES

None